Performance of outdoor seawater treatment systems for recirculation in an intensive turbot (*Scophthalmus maximus*) farm

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Abstract. Water treatment systems are mandatory in recirculating aquaculture facilities facing existing regulations, but data on system efficiency, especially for marine species, are scarce. The present work aimed at contributing to the evaluation of the effluent characteristics and the performance of a combined outdoor biological and non-biological treatment system in an intensive turbot (*Scophthalmus maximus*) farm, operating under different hydraulic regimes. A preliminary study on the biofilter bacterial populations was also undertaken. Changes in effluent characteristics with pumping, season of the year and fish biomass were observed. The treatment system showed performance instability under the conditions assayed (outdoors, changeable recycle rates). Maximum removal of solids was observed in winter, with microscreen or biological filtration (up to 60%) and nitrite removal (40–98%) was achieved with ozonation. Reduction in ammonium levels was higher in summer, either mechanically (74%) or biologically (33%). Phosphate removal was higher in winter with both systems (37 and 60%, respectively). Compliance with Portuguese discharge standards was achieved. For improvements in the treatment loop, further studies on biofilter bacteria under outdoor conditions are needed, and biological denitrification is encouraged.

Introduction

Intensive fish farming produces wastes (solids and nutrients) due to fish excretion and feed losses (Pillay 1992). Worldwide regulations exist to minimize the impact of aquaculture and effluent treatment is mandatory. In Portugal, a vast legislative framework defines water quality standards as a function of its utilization, and imposes discharge limits for wastewaters (Diário da República 1998). Moreover, the installation and functioning of fish farms is subject to authorization as to the utilization of public domain waters and effluent discharges (Diário da República 2000).

Land-based marine fish farms traditionally use flow-through systems, but in coastal zones water supplies can be limited due to tidal regimes (Hussenot et al. 1998) and environmental regulations. Utilization of recirculating aquaculture systems (RAS) is encouraged and different water treatment technologies are employed (Blancheton 2000). Biofiltration is considered the most economical treatment system for aquaculture (van Rijn 1996) and different biofilter configurations exist. Nevertheless, due to construction, operation and economical feasibility problems, their use is common only in hatcheries or nurseries cultivating high value fish (Avnimelech 1998). These production facilities have constant water recycle rates and constant high water temperature and use small to medium scale indoor water treatment systems (Blancheton 2000). For large-scale, grow-out, cold seawater systems, few commercial applications are known. Work developed so far applies mainly to eels, as marine fish seem to be more sensitive to cultivation in fully recycled water (Blancheton et al. 2002). Therefore, for the sustainability of existing marine coastal aquaculture, it is necessary to use and improve partial water reuse systems.

Field data on large-scale effluent treatment systems are scarce for land-based intensive turbot production (Hussenot et al. 1998; Borges and Soares 2001; Blancheton et al. 2002). Moreover, references to marine fish farms working under different cycles of fresh/recirculated water and using outdoor large-scale treatment systems involving biological and non-biological processes are rare. The present work aimed at contributing to the evaluation of the effluent characteristics and the performance of an outdoor biological and non-biological treatment system in a Portuguese intensive turbot (*Scophthalmus maximus*) fish farm, operating under different hydraulic regimes. In order to achieve future improvements in the biofiltration system, a preliminary study on the bacterial populations of the biofilter was undertaken.

Materials and methods

Fish farm characteristics

The private land-based turbot (*S. maximus*) farm under study was located on the Portuguese Atlantic coast. The reported annual production is 60 t. Fish were fed homemade pellets adapted to fish size. Feeding was manual, *ad libitum*, at 10.00 h (2/3 of food given) and at 14.00 h (remaining 1/3 of food). Water volume used for turbot fattening was 2600 m^3. Seawater was pumped from the shore by two pumps of 80–100 and 40 $l\,s^{-1}$ theoretical capacity each. After a storm in January 2001, only the first pump remained functioning till the end of this study. As each tidal cycle comprises two periods of high tide and two periods of low tide per day, and because tidal coefficients change bi-monthly, periods of maximum, minimum or zero pumping occurred and changed daily and weekly, with pumping maxima at high tides of spring tides. To avoid lack of fresh seawater for other operations inside the farm, a small auxiliary pump (4 $l\,s^{-1}$) was placed on the strand after the storm already mentioned, and pumped seawater continuously. Pumped seawater flowed directly to an outdoors elevated reservoir (150 m^3) and then to the indoor rearing tanks by gravity, being oxygenated before use. Effluents left each tank via central drains without solid traps and were conducted by a common drain to the outside, where treatment was carried out prior to discharge or reuse.

Implemented outdoor seawater treatment system

Untreated effluents passed through a rotating mechanical screen filter (60 μm mesh size) at the border of a concrete basin with two 90 m^3 compartments (15 m × 4 m × 1.5 m). In the first compartment, a submerged 137 m^2 biological filter was built using 950 plastic boxes (48 cm × 30 cm × 15 cm), filled with perforated plastic pieces (industrial surpluses from the box manufacture, with an average area of 50 cm^2 each). The boxes were arranged in nine horizontal double rows, 1 m apart, each row having 8–9 boxes in length and six boxes in height, placed above the ground over brick tiles. Four aeration points per double row continuously provided aeration. After crossing longitudinally the biofilter, the effluents entered the second compartment of the basin, from where they were either discharged or pumped, via two or four recirculating pumps ($2 \times 40\,l\,s^{-1}$ or $2 \times 80\,l\,s^{-1}$ respectively, depending on recycled water needs), to the ozone generation system (pure O_2, corona discharge type, 400 g ozone per hour, maximum capacity). From there, ozone was dissolved into the recycled effluent by a venturi effect in two contact columns. Treated recycled effluents then entered the elevated reservoir, where they were mixed with seawater pumped ashore. Before distribution by gravity to the fish tanks this water was further oxygenated. To maintain a constant water volume in culture tanks, the flow of water inside the farm had to be always the same, independent of tidal variations. Consequently, different percentages of recirculated water were needed.

Sampling protocol

This study involved a sampling period of 9 months, from August 2000 to July 2001. Incoming seawater was sampled directly from the offshore pumps and latter from the helping small pump mentioned earlier. Effluent was collected at the farm drain, before the treatment loop, using 10 l plastic containers. As random monthly effluent sampling, at no specific dates, did not give a clear picture of effluent characteristics (Borges et al. 2000; Borges and Soares 2001) the sampling protocol was changed attending to the season of the year (summer/winter) and the feeding situation of the fish (not fed/after feeding), but with samples taken only under optimal pumping situations (at high tides of spring tides, given by official tidal tables of Leixões Harbour, Porto, 2000–2001). Sampling dates and hours (Table 1) combined the chosen tide (high tide near full moon or new moon) and fish feeding schedule: before feeding near 10:00 h, after feeding near 16:00 h, because most of the food was given to the fish in the morning and maximum ammonia excretion is observed 5–6 h after feeding (Person-Le-Ruyet et al. 1991). Fish biomass was expected to be constant during the experimental period but due to an exceptionally strong winter, it was not possible to restock the farm in spring and, abnormally, fish stocking density decreased from 40 t in winter to 25 t in summer 2001. Additional 10 l effluent samples were collected at the same time, before and after each treatment system. As the ozonation treatment was still in an experimental phase, sampling was only

Table 1. Sampling protocol used and seawater temperature, number of pumping hours and fish biomass observed during the present work.

Sampling (dates/hours)	Fish feeding status (Bf/Af)	Seawater temperature (°C)	Daily pumping (number of hours until sampling)	Fish biomass (tons)
Summer				
August (29/08/00 – 11 h)	Bf	18.2	3.0	40
$July_1$ (10/07/01 – 11 h)	Bf	18.7	2.5	25
$July_2$ (20/07/01 – 11 h)	Bf	17.6	2.0	25
$June_1$ (11/06/01 – 16 h)	Af	18.2	4.0	25
$June_2$ (26/06/01 – 16 h)	Af	18.8	6.0	25
Winter				
$November_1$ (03/11/00 – 11 h)	Bf	13.7	10.0	35
$November_2$ (15/11/00 – 11 h)	Bf	12.5	6.2	39
January (17/01/01 – 16 h)	Af	12.3	6.0	40
March (28/03/01 – 16 h)	Af	14.2	7.0	42

Bf – Before feeding
Af – After feeding

possible when the ozone generator was on. Due to various logistic problems, the predicted weekly and 24 h sampling cycles were not feasible. Also sampling beyond normal working hours was not possible. Nevertheless, one full working day sampling was performed using the farm laboratory facilities. Data on farm management on the sampling days was obtained from the farm biologist.

Analysis performed

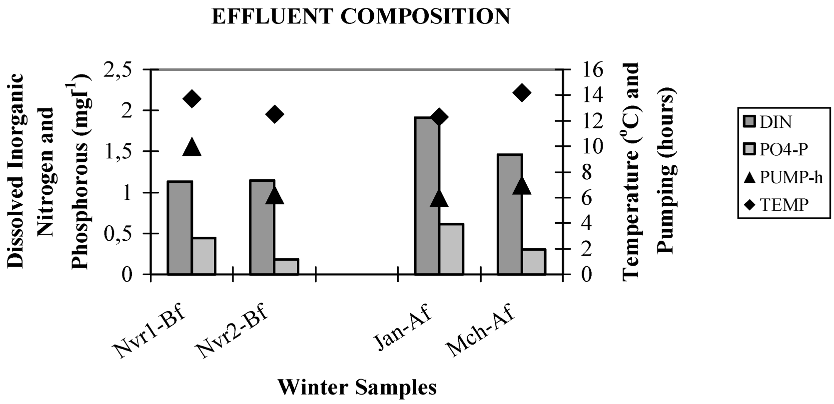
Collected samples were treated according to Aminot and Chaussepied (1983): filtration by Whatman GF/C fibreglass filters and immediately analyzed in triplicate or kept frozen ($-18\,^{\circ}C$), for 1 week to 1 month, until analysis of the parameters nitrogen (as total ammonia nitrogen, TAN, $N\text{-}NH_{3,4}$ also referred in this work as $N\text{-}NH_4$ or ammonium, nitrite – $N\text{-}NO_2$ and nitrate – $N\text{-}NO_3$) and phosphorous (as phosphate – $P\text{-}PO_4$). Total suspended (TSS) and volatile suspended (VSS) solids were analyzed according to APHA (1992). Data collected *in situ* referred to temperature (digital thermometer), salinity (refractometer YSI instruments), dissolved oxygen (Oxyguard oxymeter) and pH (OAKLON portable meter). Ozone monitoring was carried out by the farm biologist using a specific probe for ORP evaluation, and chemical tests for bromine determination (Palintest test kits). Treatment system efficiency (E) was calculated from water samples taken before (conc. before) and after (conc. after) each existent treatment unit, according to the formula: $E\ (\%) = [(\text{conc. before} - \text{conc. after})/\text{conc. before}] \times 100$.

Samples for enumeration of free and fixed bacteria were taken in spring, from the biofilter central region. Culturable attached bacteria were recovered by scraping the surface of three pieces of the biofilter plastic filling and vortexing the material at maximum speed for 30 s. The extracted biomasss was revivified in 100 ml saline yeast extract medium (Leonard et al. 2000). Enumeration of viable marine and non-marine heterotrophic bacteria (CFU) was made by plating diluted suspensions (0.1 ml) onto marine agar (Difco 2216) and nutrient agar (LabM), and incubation for 4–7 days at 25 °C. Isolation of bacteria capable of utilizing nitrate under anaerobic conditions (possible denitrifying bacteria) was carried out using nitrate agar, with and without NaCl, prepared according to Rhee et al. (1997). Samples of diluted suspensions were spread onto replicate plates and incubated in anaerobic jars with CO_2–H_2 gas-generating system (Anaerocult); the existence of an anaerobic atmosphere was confirmed by the reduction of resazurine indicator strips (Oxoid-anaerobic indicator-BR 55). The incubation was done at 25 °C for 4–7 days. A preliminary characterization of selected isolates was based on Gram-staining, colony and cell morphology, colour, presence or absence of cytochrome c oxidase and catalase. Gram-negative isolates were further identified using the API 20 NE system (Biomerieux), and the software program Apilab Plus (Biomerieux).

Results

Pumped seawater and effluent characterization

Good quality seawater entered the fish farm, as nutrient and solids concentrations were within the values observed by Aminot and Chaussepied (1983) for unpolluted coastal waters (values below 10^{-2} mg l^{-1} for nutrients and 1–3 mg l^{-1} for solids). Nevertheless, nitrate levels, with summer maxima of 0.58 ± 0.01 mg l^{-1} offshore and 3.14 ± 0.09 mg l^{-1} near the beach, tended to be higher than those found in normal coastal waters, a fact which may be due to coastal runoff. Untreated effluents showed higher nitrogen and phosphorous contents when compared to incoming seawater. Average nutrient concentration values (winter-before/after feeding and summer-before/after feeding) varied between $0.39 \pm 0.01/0.69 \pm 0.43$ and $2.66 \pm 2.51/2.84 \pm 1.42$ mg l^{-1} for N-NO_3, $0.22 \pm 0.05/0.15 \pm 0.04$ and $0.55 \pm 0.12/0.34 \pm 0.23$ mg l^{-1} for N-NO_2, $0.53 \pm 0.04/0.85 \pm 0.16$ and $1.94 \pm 1.36/1.08 \pm 0.45$ mg l^{-1} for N-NH_4 and from $0.31 \pm 0.19/0.46 \pm 0.22$ to $1.54 \pm 0.46/1.01 \pm 0.42$ mg l^{-1} for P-PO_4. Minimum levels were always found in winter and maximum levels in summer. For TSS, this trend was not observed, with $9.69 \pm 2.11/19.85 \pm 7.67$ mg l^{-1} in winter (close to the maximum seawater value of 19.65 ± 0.90 found in November$_2$) and $7.20 \pm 1.84/9.10 \pm 0.85$ mg l^{-1} in summer. During the present study, the temperature of the pumped seawater varied between 12.3 °C in winter and 18.8 °C in summer, oxygen levels varied between 9 and 13 mg l^{-1}, salinity between 30 and 34‰ (offshore values; in water pumped near the beach salinities from 18–20‰ were observed) and pH varied between 7.8 and 8.2. Values observed in untreated effluent for the parameters referred above closely resembled those of seawater, with the exception of pH, which varied between 6.4 (summer) and 7.6 (winter). Although sampling was done during pumping at high



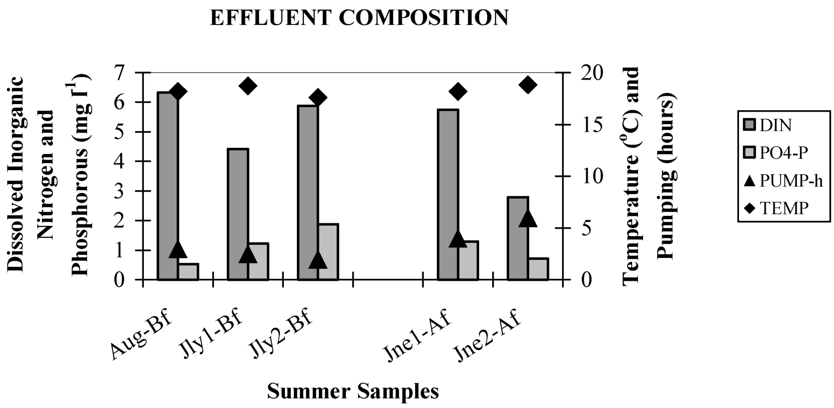


Figure 1. Untreated effluent characteristics observed under different conditions of temperature, feeding regime and pumping. Data presented according to season, year of sampling (2000–2001) and fish situation (before feeding – Bf; after feeding – Af). Fish biomass changed from 25 t in summer (except August 2000 with 40 t) to 40 t in winter.

tides, because of monthly and annual changes in tidal coefficients, the number of pumping hours observed from the beginning of the day of sampling (00 h) till the moment of sampling changed too, being higher in winter and lower in summer (Table 1).

From Figure 1 it can be seen that effluent nutrient composition changed with season, being higher in summer, despite the lower biomass reared. This fact was probably related to the combination of high temperature (increasing excretion rate) and low pumping (low water renewal rate) observed. In a situation where the fish load was the same in both seasons (August/2000/Bf v.s. winter, with 40 t) summer nutrient values continued to be higher than winter ones, and of equivalent charge when compared to the other summer samples with lower biomass, which agrees with the presumed importance of hydraulic regimes in this type of farm. Under the same pumping regime (6 h) and feeding status (Af) the effect of summer temperature prevailed over the effect of winter biomass, with higher DIN (the sum of all inorganic nitrogen forms) and phosphorous values in summer. The effect of fish feeding status (not fed/after feeding in each season) in the effluent water quality was not clear, especially in summer samples and to show the variations observed, replicated data of each feeding situation in the same season were not averaged in the figure. These results could be attributed to an insufficient time-lapse for after feeding samples or to the effect of water renewal (pumping), as temperature and biomass (excluding August) were constant within each seasonal group (Table 1).

Treatment system efficiency

A comparison between the effluent composition before and after one single passage by each component of the treatment chain (Figure 2) showed that the treatment was not always efficient. For treatment and reuse purposes only the ozone generator enabled consistent and positive efficiencies for nitrite removal (maximum of 98% in summer and 40% in winter). Without ozonation, N-NO_2 levels tended to rise up to 46% more than the levels for the untreated effluent. However, increases in the levels of ammonium, nitrate and phosphate after effluent ozonation were sometimes observed. Removal of solids by ozone treatment was observed only once, in summer, and with low efficiency (6%). Data on effluent bromine levels and ORP after ozonation showed normal functioning of the ozone generator. The existing outdoor mechanical and biological filters showed inconsistent results for solids removal and nitrification, changing from their effective removal to addition to the effluent. Nevertheless, mechanical filtration reached a maximum of 74% ammonium removal in summer, and 44% nitrate and 37% phosphate removal in winter. Solids were also effectively removed in winter (maximum of 65% for TSS and 67% for VSS). As to nitrite, removal was observed only at one sampling stage carried out in July (10% efficiency). On the other hand, the biological filter was efficient throughout the sampling stages for particle removal (up to 61% for TSS and 64% for VSS in winter) and for ammonium removal in summer (maximum of 33%). Nitrate was also more easily removed in summer (efficiency up to 72%) and phosphate in winter (maximum efficiency of 60%). Nitrite usually increased after one single passage of the effluent through the biofilter and a positive removal of only 7% was registered in winter.

Results obtained during a one-day stay in the fish farm (month of April, 15 °C, 90% calculated recycle rate) confirmed the need for ozonation for effluent nitrite reduction (63% efficiency observed only after 3 h of continuous operation) and the importance of the biofilter for ammonium depuration (17% efficiency).

The existing Portuguese legislation reference values for seawater quality for specific uses (Diário da República 1998, Decreto-Lei 236/98, 1 Agosto – Water

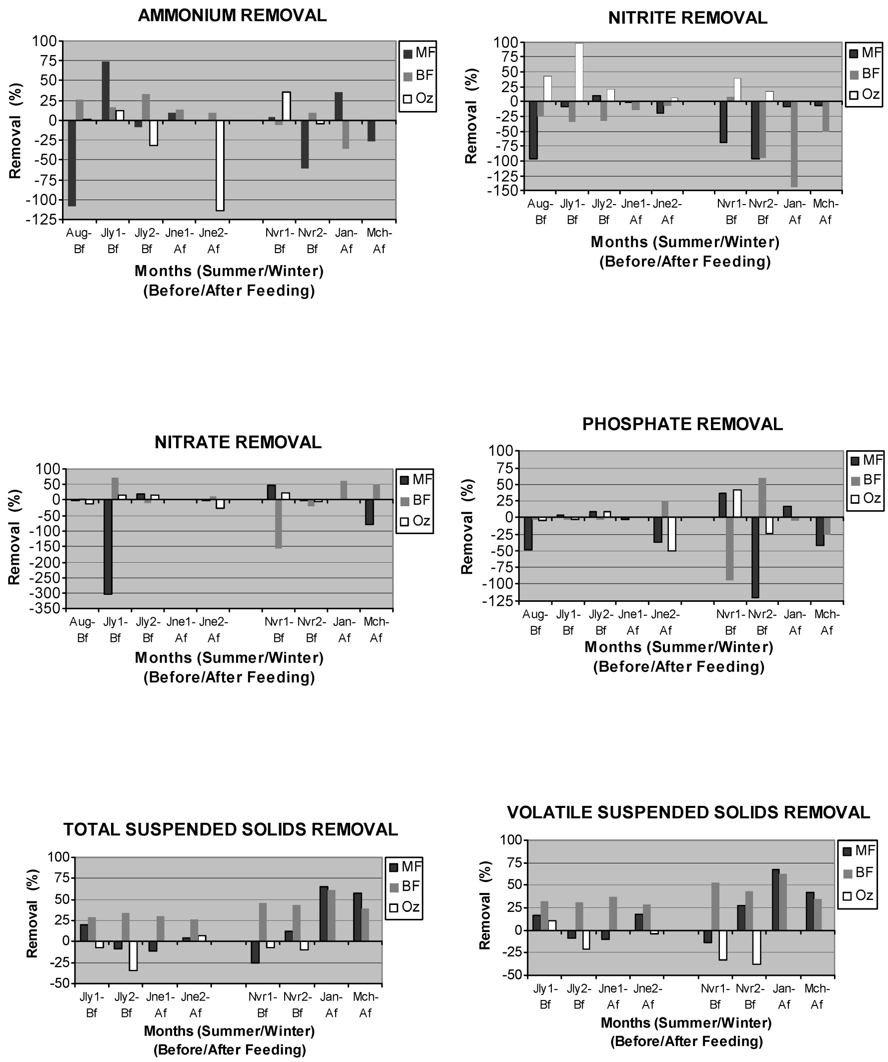


Figure 2. Efficiency of outdoor effluent treatment system (FM – Mechanical Filter; FB – Biological Filter; Oz – Ozone Generator). Negative values represent an increase in the level of the parameter after the correspondent treatment unit. Data arranged in two groups according to season (summer/winter), sampling year (2000/2001) and fish feeding status (before/after feeding).

Quality Law) and standards for discharge of urban wastewaters (Diário da República 1997, Decreto-Lei 152/97, 19 Junho) are shown in Table 2. It must be stressed that Portuguese coastal waters (with the exception of Algarve) are considered non-sensitive zones as to eutrophication risk and therefore water quality criteria for urban wastewater discharge are not in place for the parameters N and P.

Table 2. Obtained and available reference values for studied variables.

Parameters	Seawater quality standards[1]		Wastewater discharge maximum values[1,2]	Treated turbot effluent values[3]	
	Shellfish cultivation	Bathing		Maximum	Average
pH	7–9	6–9	6–9	7.6	6.9
Oxygen (%sat; $mg\,l^{-1}$)	>80%	80–120%	BOD5 = 40; 25 COD = 150; 125	13	12
TSS ($mg\,l^{-1}$)	<30% change from original value	Not referred	60; 35–60 (or 150 from lagoons)	25.2	11.5
N-NH4 ($mg\,l^{-1}$)	Not referred	a	10	2.9	1.1
N-NO2 ($mg\,l^{-1}$)	Not referred	Not referred	Not referred	0.63	0.30
Total N ($mg\,l^{-1}$)	Not referred	a	15; 10–15[b]	nd	nd
N-NO3 ($mg\,l^{-1}$)	Not referred	a	50	5.2	2.0
P-PO4 ($mg\,l^{-1}$)	Not referred	a	Not referred	1.9	0.8
Total P ($mg\,l^{-1}$)	Not referred	Not referred	0.5, 3, 10; 1–2[b] depending on receiving waters	nd	nd

Sources: 1 – Decreto-Lei 236/98, 1 Agosto; 2 – Decreto-Lei 152/97, 19 Junho; 3 – Present study.

a – Parameters to be analyzed by governmental authorities whenever there is a tendency for eutrophication of receiving waters.

b – Values for urban wastewater discharge in sensitive zones, with eutrophication risk.

nd – not determined.

It can be seen that the treated turbot effluent values can be generally considered within the existing Portuguese water quality standards for wastewater discharge and common coastal seawater uses (shellfish cultivation and bathing).

Analysis of the biofilter bacteria

The results of the preliminary study on biofilter fixed bacteria showed a higher morphological bacterial diversity when nutrient agar was used, with 13–15 morphologically different colonies recovered, compared with six colony types recovered when marine agar was used. In the seawater crossing the biofilter (free bacteria), 5–6 morphological colony types were recovered using marine agar and bacterial populations were found in the order of 2.1×10^5 CFU ml^{-1}. Fixed bacteria population recovered from the biofilter surface at the same sampling point were in the order of 8.6×10^4 CFU cm^{-2}. The use of NO_3-agar (with or without salt added) under anaerobiosis resulted in the recovery of only one morphological colony type, found in the order of 1.6×10^4 CFU cm^{-2}. The isolation of the morphologically different colonial types recovered from the biofilter and identification by API revealed that those belonged to the Proteobacteria, subdivisions α, β and γ: *Shewanella putrefaciens* (identification probability: 89%), *Agrobacterium radiobacter* (identification probability: 99.9%), *Chryseomonas luteola*/*Shphingomonas paucimobilis*/*A. radiobacter* (low discrimination) and included a NO_3-user isolated under anaerobiosis, *Burkholderia cepacia* (identification probability: 92%). The isolation of the morphologically different colonial types recovered from water (free bacteria) revealed the presence of two different bacteria: *S. paucimobilis*/*C. luteola* (low discrimination) and *S. putrefaciens*/*Aeromonas salmonicida masoucida*/*Achromogenes* and *Pseudomonas vesicularis* (low discrimination). No Vibrionaceae were found under the environmental conditions prevailing at the time of sampling (spring samples, seawater temperatures of 12–14 °C, 28–29‰ salinity and pH = 7.0–8.0).

Discussion

Results obtained in the present work are within existing data for intensive fish farm effluents, presenting higher nutrient values when compared to seawater (Hussenot et al. 1996; Lemarié et al. 1998; Tovar et al. 2000). It is also clear that untreated effluent composition was affected by several factors and that existing data are not enough or appropriate for multivariate statistical analysis. Besides, some experimental factors, like fish biomass, could not be controlled as predicted and the attempt to fix sampling to periods of maximum pumping did not avoid the effect of tidal coefficient variations on the volume of water pumped. Despite these constraints Spearman rank order correlation analysis (rs, Siegel and Castellan 1989) was used to assess possible associations between the variables temperature, biomass, pumping and nutrients. A significant correlation was found between biomass and temperature after the exclusion of August and $June_2$ samples ($rs = -0.61$,

$p = 0.02$); thus, any conclusions on the effect of these two parameters on effluent characteristics must be drawn with caution. No correlations were found between pumping and temperature or biomass (rs = 0.51, $p = 0.16$) but significant associations were obtained for pumping with all nutrients assayed (rs = −0.73, $p = 0.03$ for ammonia, rs = −0.70, $p = 0.04$ for nitrite, rs = −0.80, $p = 0.01$ for nitrate and rs = −0.80, $p = 0.01$ for phosphate). Effluent suspended solid were not associated with this factor (rs = 0.33, $p = 0.42$). Summer effluent ammonium levels were usually higher than in winter, which may be an effect of temperature, which is positively related to fish excretion (Burel et al. 1996). Nevertheless, the influence of temperature under changing hydraulic situations should be clarified and the development of predictive models relating daily TAN production, temperature and pumping would be useful. The maximum TAN values found ($2.9\,\mathrm{mg\,l^{-1}}$ in summer, at 18.7 °C and pH < 7.0) imply minor toxicity risks to fish (NH_3 fraction ≈ $0.01\,\mathrm{mg\,l^{-1}}$). However, a small increase in pH values (i.e., biofilter nitrification failure or increased denitrification) can raise the NH_3 values to concentrations above $0.04\,\mathrm{mg\,l^{-1}}$, which may induce sub-toxicity effects in marine fish (Hussenot et al. 1996; Losordo et al. 1998). Changes in seawater renewal rates (pumping) should be further analyzed and higher effluent nutrient levels are expected under lower pumping capacities. This might affect not only the efficiency of seawater treatment systems but also effluent compliance with legislation.

Open-flow fish farms present a seasonal effect concerning suspended solid concentrations, with maximum values registered in summer, when feeding and fish growth are highest (Tovar et al. 2000). This trend was not observed in the present study, may be due to the different fish biomass reared in the two main seasons (high in winter, abnormally low in summer) and to the increase in solids observed in incoming seawater during winter storms. Solids in aquaculture systems can decrease water quality and increase fish stress. They are usually removed mechanically by microscreens, with 60 µm mesh sizes removing particles with efficiencies varying from 67–97%, depending on waste effluent concentrations, which, in turn, depend on pre-treatment techniques applied (Cripps and Bergheim 2000). In the present study, maximum efficiencies for TSS removal of 65% in winter agree with these values, despite the fact that no solids pre-treatment was employed. The filter showed lower efficiency in summer, perhaps due to the low biomass cultivated at this time. Microscreen filtration was sometimes efficient for the removal of dissolved ammonium (maximum of 74% in summer and 35% in winter), dissolved phosphorous (maximum of 37% in winter and 10% in summer) and nitrate (maximum of 44% in winter and 20% in summer). This might be related to adsorption of these nutrients to the particulate fraction of the effluent (Bergheim et al. 1993). The use of cost-effective water treatment systems is vital for RAS (Losordo et al. 1998) and biofiltration is considered the most appropriate solution for dissolved nitrogen removal (van Rijn 1996). Biofilters are widely used and nitrification is dependent on successful competition of nitrifiers for space and nutrients. The biofilter studied was an outdoor homemade version of a submerged filter, with tangential flow of water and no backwashing. Its filling material prevented expansion of the filter bed by water flux. Thus, the filter structure served as a baffle, diminishing water velocity and increasing solids sedimentation, a phenomenon common in biofilters (Nijhof and Bovendeur 1990). In fact, throughout the year, efficient solids removal was observed: 39–61% in winter and 27–34% in summer for TSS, 63–35% in winter and 29–37% in summer for VSS. High solids deposition, increasing organic matter and decreasing oxygen availability, might have affected biofilm development and functioning with faster growing bacteria (e.g., heterotrophs) being promoted and nitrifying bacteria depressed. This effect would have been enhanced by high water flow rates, low temperature, low pH (possibly also resulting from CO_2 production by heterotrophic bacteria) and low dissolved oxygen (3–4 $mg\,l^{-1}$ in biofilter outflow), and thus ammonium and nitrite accumulated in the outlet in winter. Photoinhibition phenomena (Hagopian and Riley 1998) were not likely to have occurred due to the shading of the biofilter surface with dark screens.

The utilization of ozonation in the treatment loop supports the findings of Otte and Rosenthal (1979) and Summerfelt et al. (1997) that high nitrite levels can be avoided by ozonation of aquaculture recycled water. Nevertheless, the beneficial effect of ozone on solids removal, especially due to microflocculation of colloidal organic matter, was not generally observed. Also, a tendency for VSS increase after ozone treatment was registered, possibly resulting from oxidation of low-biodegradable organic compounds (Summerfelt et al. 1997). A decrease in TAN following ozonation of wastewater is commonly expected (Krumins et al. 2001) but was not always observed in our study. In ozonated water increases in phosphorous (released from colloids and humic substances) and of nitrate concentrations have been reported (Wheaton 1977; Lehtola et al. 2001). This might have occurred also in our system for phosphorous but data on nitrate levels were not conclusive. The ozone treatment system was still under testing and the results obtained cannot be considered definitive. Also, performance could have been negatively influenced by the placement of the ozone generator after the biological filter (Krumins et al. 2001; J.-P. Blancheton, personal communication).

Very little has been published on non-pathogenic bacteria, including nitrifiers, ammonifiers and denitrifiers, in RAS studies under operating outdoor marine biological treatment systems (Hagopian and Riley 1998; Leonard et al. 2000). A bacterial species identified as *S. putrefaciens*, a heterotrophic bacterium close to the ammonia and nitrite oxidizers (Hovanec and Delong 1996) was recovered from the biofilter under study. *B. cepacia* was also recovered, using an enriched nitrate medium under anaerobiosis. This is an interesting finding, as Mullan et al. (2002) found that a sludge isolate of this species showed high phosphate removal and polyphosphate accumulation under mildly acidic conditions. This might be a widespread stress response and in an osmotically harsh environment such as seawater, several microorganisms probably have the same function (J. McGrath, personal communication). These aspects, linked to the need of further research on denitrification in outdoors RAS (considering the elevated nitrite effluent values observed and the high prices of ozonation), open interesting doors for applied bacterial research.

Conclusions and recommendations

Despite the fact that fish farms are dynamic production systems and therefore the previously described RAS does not correspond exactly to the present treatment system of the farm studied, some conclusions can be drawn from the work done:

1. In intensive fish farms with water availability dependent on tidal cycles, effluent characteristics seem to be strongly influenced by tidal regimes (pumping feasibility) and season of the year (temperature effects). These aspects must be considered when effluent treatment systems are being designed.
2. Conventional wastewater treatment systems perform inconsistently under changing effluent characteristics and environmental conditions. Nevertheless, the system studied proved useful for suspended solids, nitrite and total ammonia nitrogen removal and was sufficient for compliance with current Portuguese effluent discharge standards.
3. Improvements in biofiltration performance are dependent on further studies on the behaviour of heterotrophic and autotrophic attached bacteria under unstable (stress) conditions.
4. Although useful in RAS, ozonation of fish farm effluents is too expensive for small to medium scale producers and studies on biological denitrification are encouraged.

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